TO STUDY THE ACCURACY OF PAPSMEAR, VIA & VILI IN DETECTING PRECANCEROUS LESIONS OF CERVIX

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled "**TO STUDY THE ACCURACY OF PAPSMEAR, VIA & VILI IN DETECTING PRECANCEROUS LESIONS OF CERVIX"** is a bonafide record work done by **Dr. S. AARTHY** under my direct supervision and guidance, submitted to the Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of University regulation for M.D Branch II – Obstetrics & Gynaecology.

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DECLARATION

I **Dr. S. AARTHY** solemnly declare that the dissertation titled **" TO STUDY THE ACCURACY OF PAPSMEAR, VIA & VILI IN DETECTING PRECANCEROUS LESIONS OF CERVIX"** has been prepared by me. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any other University board either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulation for the award of M.D degree Branch – II (Obstetrics & Gynecology) to be held in March 2009.

Place : Madurai **Dr. S. AARTHY**

Date :

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CONTENTS

INTRODUCTION

An estimated 4,70,000 new cases of cervical cancer are diagnosed each year worldwide. 80% of these occur in developed countries.

A quarter of the global burden is experienced in India, where about 1,26,000 new cases &71,000 deaths attributable to cervical cancer are estimated to occur each year.

Cancer cervix constitutes 15-51% of all the female cancers and rate of age standardized incidence range from 17.2 to 55 per 1,00,000 women in different regions of India.

More than 80% of cases are diagnosed at an advanced clinical stage and five year survival is less than 40%.

In many developed countries a decline in the incidence of and mortality caused by cervical cancer has been observed in the past 30 years as a result of screening cytology. Screening programmes do not exist in many developing countries, however and in some low resource settings, where cytology programme do exist they have not been effective in reducing the disease burden.

Due to difficulties in ensuring high quality cytology services in many settings, there have been significant interest in new approaches of screening for precancerous lesions.

Of these, visual inspection of cervix is a promising option, especially for low resource settings.

Visual inspection with acetic acid, which identifies acetowhite lesions, has now been recognized as one of the promising methods for cancer detection from Indian & African studies as compared to cytology under similar circumstances.

Analysis from a growing number of studies in developing country settings indicate that the sensitivity of VIA is equivalent to or greater than Cytology, although its specificity is somewhat lower.

Other forms of visual inspection with Lugol's Iodine (VILI) is similar to the Schiller's Iodine Test used in the 1930s and has been reevaluated in recent studies as an alternative for use in low resource settings.

The largest set of pooled data from IARC multicentre study of VILI indicates that it is more sensitive than or equally specific to VIA.

Many aspects of VIA &VILI make them appearing for use in low resource settings, as compared to Pap smear because of similar approaches which do not require laboratory involvement. Furthermore non-Physicians can perform the procedure with adequate training. Also visual methods are relatively simple and inexpensive. The results are available immediately.

AIM OF THE STUDY

The aim of the study is

To calculate the accuracy of Papsmear, VIA, VILI in detecting precancerous lesions of cervix, reference standard being biopsy cervix.

To calculate the sensitivity, specificity, positive predictive value, negative predictive value of these three tests and to compare the results.

REVIEW OF LITERATURE

Historically before the advent of papsmears and programmatic screening, health care workers relied on looking at the cervix to detect abnormalities.

Schiller's test has been used for many years to aid in differentiating mature normal epithelium from immature abnormal epithelium.

After 1950s, when pap smear became the standard for cervical cancer screening many women were referred to colposcopy to confirm screening finding.

Increasing number of women undergoing this test led to increased utilization of the colposcopy (initially developed in 1930s) to confirm screening findings. Years later, given the expense and expertise of colposcopy, clinicians began to explore whether unmagnified visualization of the cervix could be used as an adjunct to cytology so that patients in need of colposcopy could be identified more effectively and efficiently.

Also papsmear was found to have several short comings. Papsmear quality and coverage are low. In addition, even in middle income countries programme organization to adequately manage, treat and follow up women with identified lesions can be a challenge (Sankaranayanan et al 2001). The WHO Reproductive Health Library

includes a summary of a meta analysis of pap test accuracy that reviewed 62 studies and concluded “the paptest may be unable to achieve concurrently high sensitivity and specificity” (Fahey 1995). To address these limitations, a number of new cervical cancer screening approaches are being evaluated, including visual inspection, automated Pap screening, alternative approaches to specimen collection, and protocols using HPV tests.

Four screening techniques based on visual inspection have been assessed for early detection of cervical neoplasia in low resource settings

- Unaided visual inspection (also known as downstaging)
- Visual inspection with 3-5% acetic acid (VIA)
- Visual inspection with acetic acid under magnification (VIAM)
- Visual inspection with lugol’s iodine (VILI)

Unaided visual inspection (also known as downstaging)– involves naked eye inspection of cervix without application of acetic acids to identify abnormal cervical tissue harboring cervical neoplasia , particulary invasive cancer. Cross sectional studies in India have shown low sensitivity for visual inspection to detect cervical cancer precursors and it is no longer considered a suitable screening test (Sankarnarayanan et al, 1997, Basu et al, 2002).

VIAM involves the use of low level magnification (2-4x) in visualizing the acetowhite lesions after application of acetic acid. The test

characteristics of VIA & VIAM have been evaluated in cross-sectional studies in India and south Africa. (Denny et al, 2000 a, 2002), Sankaranarayan et al, 2004 e). The result from these studies indicate that magnification did not improve the test performance over and above that of naked eye visualization. Low level magnification is no longer widely used for visualization after application of acetic acid.

VIA

Involves naked eye inspection of the cervix one minute after application of 3-5% acetic acid. VIA has been widely investigated for its test characteristics in detecting, CIN 2-3 lesions and invasive cancer in several cross sectional studies, mostly in developing countries. (Londbe et al, 1997, Slarron et al, 1992, Denny et al, 2000a, Sankaranarayan et al 1999).

Ottavino et al, examined 2400 women using VIA and the colposcope ..VIA detailed abnormalities in 98.4% of patients assessed colposcopically as having an abnormal transformation zone and it correctly identified 98.9% of normal cases.

Van Le et al, found that VIA resulted in an additional 15% of CIN cases being identified among cytology negative women, but 40% of women with positive VIA underwent unnecessary colposcopy.

Slawson et al, demonstrated that VIA might be helpful in reducing referrals for colposcopy.

Frisch et al, found that combining a negative cytology and negative VIA test resulted in a negative predictive value of 90%, greater than that obtained for cytology alone, but with some loss of positive predictive value. These studies demonstrated the potential value of VIA as a viable screening approach. But did not establish its test qualities as a primary screening method.

The relative sensitivity of VIA to detect high grade precancerous lesions and invasive cervical cancer varied from 29% to 95% and specificity varied from 68% to 98% in cross sectional studies suffering from verification bias. (Slatson et al, Londhe et al, Sankaranarayan et al, 1988, 1999, Crorje et al, 2001)

In late 1990's, Londhe et al, studied 372 women who underwent VIA, cytology and colposcopy in a gynaecology outpatient clinic. VIA identified 78% of HSIL and stage I cancer, diagnosed through colpscopy 3.5 times more than those identified via cytology.

Sankaranarayan et al, 1998 studied 3000 women attending cancer detecting clinics, conducted as a part of the community outreach programs in Southern Kerala, by Regional Cancer Center, Trivandrum. They had VIA and cytology provided by trained cytotechnicians. Those

positive on one or both screening tests or those who had clinically suspicious lesions even if the tests were negative were investigated with colposcopy and directed biopsy if necessary. Those with moderate dysplasia or worse lesions diagnosed by histology were considered true positive. The detection rate of true positive cases were compared. VIA detected (90.1%)of true cases and Cytology (86.2%) of true cases yielding sensitivity ratio of 1.05. As the detection rates were similar, the study suggested further evaluation of VIA in low resource settings.

In the study by Megevand et al, in South Africa, VIA & cytology were performed in a mobile unit. About 2426 women were involved. In that setting, VIA detected 65% of high grade squamous intraepithelial lesion confirmed by reference standard.

In another study from South Africa. Denny et al, 1998 looked at the comparative performance of VIA, cytology, HPV testing, Cervicography. About 2754, women were recruited. The study showed that VIA and HPV were similar to cytology in their ability to detect HSIL. VIA however yielded more false positive. The sensitivity of VIA was 70% and specificity was 79%. The study also showed that magnification did not improve the sensitivity.

In these studies the reference investigation by colpscopy was carried out only in test positive women and a small proportion of test

negative women with the result that these studies suffered from verification bias.

University of Zimbabwe JHPIEGO 1998, cervical cancer project was the first study to yield direct estimate of sensitivity & specificity because all women testing negative or positive on screening, were offered the reference standard (colposcopy) thus avoiding verification bias. In that study about 10,934 women were screened by six trained nurse midwives at 15 primary care clinics at Zimbabwe. The sensitivity of VIA was 1.75 times higher than cytology (76.7%) versus 44.3% respectively), whereas the specificity was 1.4 times (64% versus 90.6%.) lower. The study showed that VIA is highly sensitive and could be valuable in detection of precancerous lesion of the cervix, but emphasis to increase the specificity was made.

Department of Obstetrics & Gynaecology, MAMC, New Delhi conducted a study involving 400 women to assess the sensitivity of VIA and cytology for detection of precancerous and early cancerous lesions. The concluded that VIA was very sensitive for ectocervical lesions and its immediate results help to see and treat at the first visit. The cost detection of one true lesion through acetic acid application (Rs.1699) was much lower as compared to the cost involved in cytology detected true

lesion (Rs.2227.00). They concluded that acetic acid may be a suitable low cost and feasible alternative in resource poor setting.

Basu, et al, studied, Visual inspection with acetic acid and cytology in early detection of cervical neoplasia, Kolkata, India. 5881 women aged 30-65 years were screened by VIA/VIAM/cytology. Positive cases were subjected to biopsy cervix. 18.7%, 17%, 8.2% tested positive for VIA/VIAM/cytology respectively. VIA and VIAM are more sensitive than cytology.

Tayyeb R et al, 2003, conducted a study at Gangaram Hospital, Lahore from Jan.1996 - Dec. 1999. 501 women were screened with pap smear and VIA. Positive cases were subjected to colpscopy & directed biopsy if necessary. Sensitivity of papsmear was 46.9% and of VIA was 93.9%, Specificity was 30.4% & 69.5% respectively. Accuracy of VIA was 77.5% compared to 52.8% of that of papsmear.

Five screening methods, VIA & VILI, Papsmear and HPV testing were evaluated in 11 IARC studies in Africa & India. (5 centres in India, Mumbai, Jaipur, Trivandrum, Dindigul and Kolkatta).

More than 58,000 women, aged, 25-64 years were tested with 2-5 screening tests and outcome verification was done on all women.

	Sensitivity	Specificity
VIA	83%	85%
VILI	93%	86%
Papsmear	57%	93%

The major finding from the study was the consistently higher sensitivity but equal specifity of VILI as compared with VIA.

VIA is being evaluated in three randomized intervention trials in India, to assess the reduction in incidence of and mortality from cervical cancer as compared to control group with no screening. (Sankaranarayan et al, 2003 a, b, 2004 c,d)

Sankaranarayan et al, assessed the effect of visual screening using VIA on cervical cancer incidence & mortality in a cluster randomized controlled trial in Dindugal, Tamilnadu. Women aged 30-50 years in 113 clusters in Dindugal district were randomized to VIA screening by nurses (57 cluster, 49311 women) and a control group (30,958 women). 31,343 were screened and 3088 had positive screen. Screen positive women were subjected to colposcopy / biopsy and those with CIN were treated. 20% of cancer detected at early stage were in the VIA group and 10% were in the control group. With an average of 4.95 and 5.20 years of follow up, in the VIA and control group, Cervical cancer incidence and mortality rates were lower in the VIA groups as compared to study group. A significant reduction of 25% in incidence and 35% in mortality has been observed in VIA group.

An innovative option, taking advantage of the immediate availability of test results with VIA is the Screen and Treat or Single visit

approach. The safety, acceptability and feasibility of such a single visit approach combining VIA and cryotherapy was assessed in a study in Thailand. Trained nurses tested 5999 women with VIA and 798 (13.3%) women were VIA positive. Overall, 756 women received cryotherapy, and at a one year follow up visit, the VIA test negative among treated women was 94.3%.

An IARC, multicentric cross-sectional study, comparing the test characteristics of visual inspection with lugol's iodine and visual inspection with acetic acid was conducted involving 5,200 healthy women, aged 25-59 years in kerala, India. VILI was performed after VIA and colposcopy was the reference standard. The study established that VILI has a significantly higher sensitivity than VIA, to detect CIN 2-3 lesion. (92.3% vs 73.8%). But the specificity of both tests were similar (84.0%) vs 82.4%).

To complement the growing body of evidence of the accuracy of VIA, a recent study examined the reliability of VIA. The data showed moderate to substantial interrate reliability for clinician assessment of cervical photographs taken after using the acetic acid wash. This reliability statistics is comparable to similar tests of interrates agreement for colposcopy, cervical cytology and histology

(Sellors et al, 2002) and is a significant step towards validating the accuracy of the test.

Researches have also been developing mathematical models to evaluate the cost benefit of alternative screening strategies such as VIA. In a model based on data from Thailand, VIA saved the greatest number of lives and was associated with the least costs, when used to screen women between the age of 35-55 years at five years intervals and coupled with immediate treatment of positive results. (Meadel blatt et al 2002). Furthermore, in a study simulating conditions in South Africa, VIA followed by immediate cryotherapy is associated with projected 26% reduction in incidence and was found to be economical in comparison to no screening.

The study conducted by division of Cancer Epidemiology & Genetics – National Cancer Institute, Maryland, USA involving 1921 asymptomatic women compared the efficacy of VIA and papsmear as a screening modality for detecting cancer cervix. They concluded that not only in low resource setting, but also in well equipped health center VIA is a good screening modality.

Test performances of various modes of screening in different studies

AUTHOR	Cytology		VIA		VIAM		VILI	
	vitySensiti	citySpecifi	vitySensiti	citySpecifi	vitySensiti	citySpecifi	vitySensiti	citySpecifi
De Vuvst et al 2005 (7)	83.3	94.6	73.3	80.0	-	-	-	-
Goel et al 2005 (8)	50.0	97.0	96.7	36.7	-	-	-	-
Ghaemmmaghami et al (2004), (9)	72.0	90.2	74.3	94.0	-	-	-	-
Sankara narayanan et al, 2003 (15)	72.1	91.6	93.4	85.1	-	-	-	-
Singh et al, (12)	73.3	99.0	86.7	94.3	-	-	-	-
Lancet 1999 (13)	44.3	90.6	76.7	64.1	-	-	-	-
Sankara narayanan et al, 1998(14)	86.2	91.3	90.1	92.2	-	-	-	-
Shastri et al 2005 (6)	57.4	98.6	56.9	88.4	64.9	86.3	75.4	84.3
Basu et al 2003 (10)	29.5	92.3	55.7	82.1	60.7	83.2	-	-
Sankara narayanan et al, 2003 (11)	81.9	87.8	82.6	86.5	-	-	87.2	84.7
Parashari et al, 2000 (20)	78.9	99.0	-	-	82.9	94.3	-	-
Sankara narayanan et al, 2004 (18)	-	-	76.8	85.5	-	-	91.7	85.4
Sankara narayanan et al, 2004 (19)	-	-	60.3	86.8	64.2	86.8	-	-
Denny et al, 2002 (16)	-	-	70.0	79.0	74.0	77.	-	-
Belinson et al, 2001 (21)	-	-	71.0	74.0			-	-
Winkler et al 2003 (17)	-	-			60.0	69.0	-	-

CIN

The epidemiological data correlate well with the current understanding of the pathogenesis of cervical neoplasia. The incidence of cervical dysplasia is reported to be 15:1000 in women who were cytologically screened. The incidence of severe dysplasia is reported to be 5:1000 according to ICMR studies. (SHAW)

The period of early squamous metaplasia is the time of greatest risk for cellular transformation and development of cervical neoplasia.In this period young metaplastic cells have phagocytic properties and if some potent mutagen is present in the vagina during this time, epithelium might undergo the cellular transformation. The early squamous metaplasia occurs almost exclusively in puberty and early adolescence and in the first pregnancy. Therefore women who begin sexual life at an early age when the metaplastic process is more active, have a greater chance of developing cervical carcinoma.

Age adjusted incidence rates for invasive cancer ranges from 19-44/100000 women in various cancer registries in India. Higher incidences noted in Chennai 43.5/100000 followed by 30.1/100000 at Delhi and 19.4/100000 at Mumbai.

The cumulative risk of CA cervix is 1.58% in Mumbai and 3.6% in Chennai. In other words, 1 in 62 women in Mumbai and 1 in 28 women in Chennai may develop cancer cervix in their life (ICMR-1992)

Natural history of CIN

Original squamous epithelium is derived from the congenital sinus epithelium, starts at the vulvovaginal line, lines the vagina, covers the major portion of the cervix and abuts upon the columnar epithelium to form the original squamocolumnar junction.

Under low vaginal acidity, the reserve cells proliferate, lifting the columnar epithelium. There is centripetal growth of original squamous epithelium growing beneath the columnar epithelial cells. Process of squamous metaplasia begins in the lips of the columnar villi which are first exposed to vaginal acidity.

A new squamocolumnar junction is continuously formed that gradually replaces the native columnar epithelium .The deeper clefts, however, may not be completely replaced by metaplastic epithelium, traped under the squamous epithelium resulting in the formation of nabothian cysts. Gland openings and nabothian cysts mark the original squamocolumnar junction and the outer edge of the transformation zone. The area between original squamocolumnar junction and the new original squamocolumnar junction is referred as Transformation Zone (TZ)

Physiological transition occurs during 3 phases of life.

1. Foetal
2. Menarche
3. First Pregnancy

Anatomy of Transformation Zone

Proximal border of Transformation Zone is the upper limit of squamous metaplasia where the immature squamous metaplasia abuts a circumferential ring of unaltered columnar epithelium.

Original squamocolumnar junction has 4 layers.

1. Basal layer: Single row of immature cells, with large nuclei and small cytoplasm.

2. Parabasl layer: Two to four rows of cells that have normal mitotic figures.

3. Intermediate layer: Includes four to six rows of cells with large amount of cytoplasm in a polyhedral shape separated by intercellular space.

4. Superficial layer: Includes five to eight rows of cells with small uniform pyknotic nuclei and acidophilic cytoplasm filled with glycogen. These cells exfoliate and form the basis for Pap smear.

Columnar epithelium

Single layer of columnar cells with mucus at the top and a round nucleus at the base.

Metaplastic epithelium :

Transformation from Columnar epithelium to squamous epithelium is known as metaplasia to the histologists and as TZ to the colposcopists.This is the area of development of neoplasia and the area of interest to the colposcopists. It's caudal limit is the original squamocolumnar junction and cephalic limit is the new squamocolumnar junction.

Pathogenesis of CIN

In most cases, CIN is believed to originate as a single focus in the transformation zone at the advancing SCJ. The anterior lip of cervix is twice as likely to develop CIN as the posterior lip and CIN rarely originates in the lateral wall.

Once CIN occurs, it can progress horizontally to involve the entire TZ, but usually does not replace the original squamous epithelium. Proximally CIN involves the cervical clefts and the area tends to have more severe lesions.

CIN is most likely to begin either during menarche or after first pregnancy, when metaplasia is more active. Conversely, a women who has reached menopause without developing CIN has little metaplasia and is at a lower risk.

Progression and regression of CIN

The spontaneous regression rate of biopsy proven CIN I is 60-85% in prospective studies. This regression typically occurs within a 2 year follow up with cytology and colposcopy.

This information has led to the recommendation that patients who have biopsy diagnosis of CIN I with satisfactory colposcopy and who agree to the evaluation every 6 months can be treated by observation. If the lesion progresses in follow up, persistent after 2 years, ablation is recommended (Novak's gynaecology)

Etiology and risk factors

1. Marriage and sexual behaviour

Increased risks are associated with the coitus at an early age, coitus with multiple sexual partners, and coitus with high risk men (Miller et al 1976, Canadian Task FORCE -1982)

2. Local hygiene

Cancer cervix is found to be positively associated with lack of daily genital washing and negatively with the use of clean sanitary napkins during menstruation (Zhang et al.1989)

3. Contraception

Barrier methods may be protective. But OCP increases the risk of CIN and invasive cancer which is attributed to the hormonal changes produced in the cervical epithelium. (Beral et al, 1988)

4. Dietary factors

Deficiency of Vit A, C, E and Folic acid are related to cancer cervix and dietary supplementation of these vitamins may prevent its occurrence (Verrult, 1989)

5. Smoking and douching

Smoking and douching with tar substance may have a role in CIN (Winkelstein et al, 1991).Smoking more than 10 cigarettes per

day had increased the relative risk to get CIN and invasive cancer (Nunez et al, 2002)

6. STD

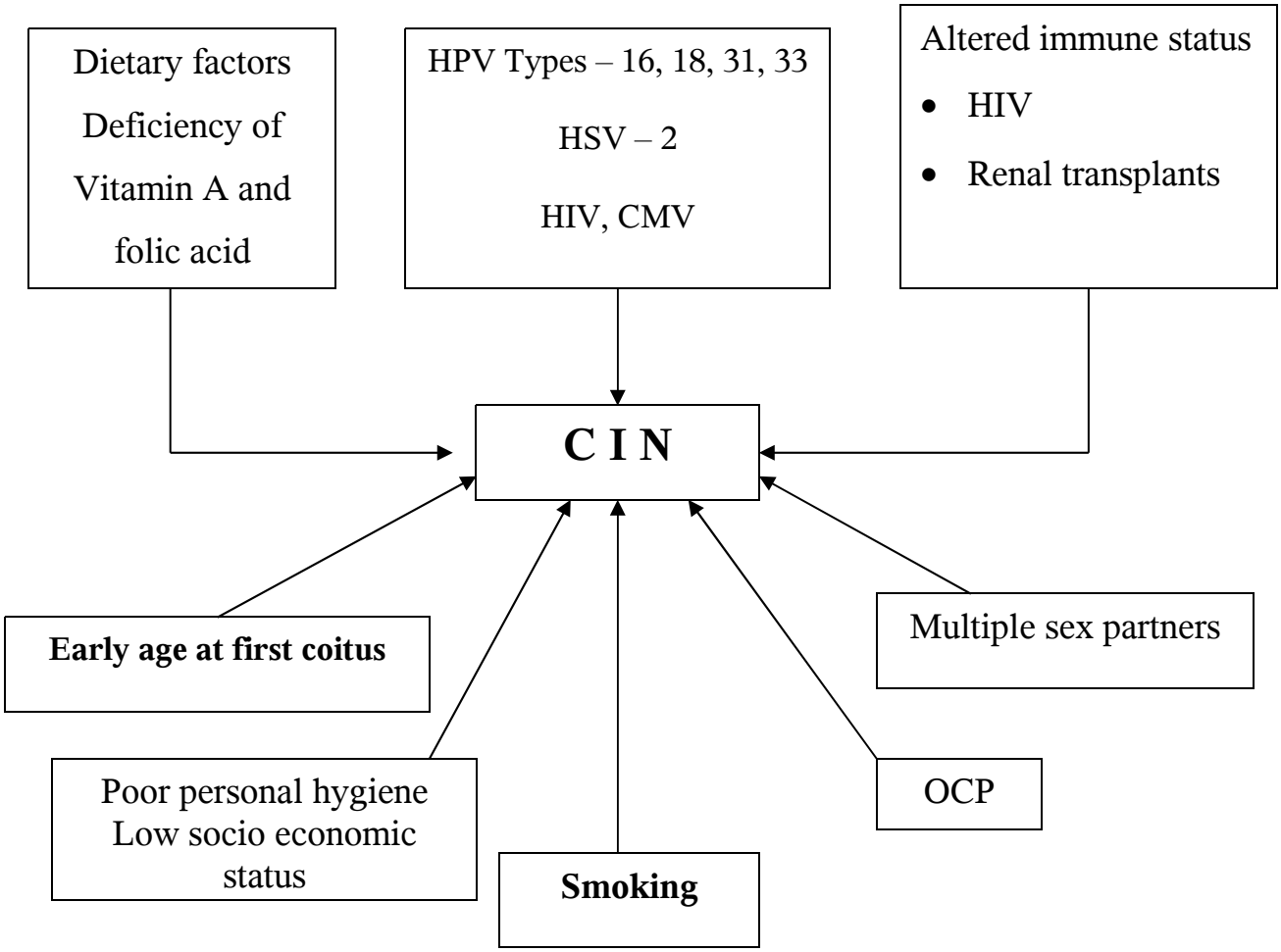
There is a definitive association of Human Papilloma Virus (HPV)-16,18,31,33 with CIN. HPV-16 is associated with CIN. HPV-16 is associated with high grade squamous cell carcinoma and HPV-18 is associated with adenocarcinoma, HPV -31,33,35 are associated with intermediate oncogenicity (Crum et al,1985.Levine ET AL,1984). Increased risk of cervical cancer has been reported with other STD infections like HSV-2, HIV, CMV. This risk is found to increase significantly with increase in the number of apparent organisms. (Schmauz et al.1989)

7. Immunosuppression

Both preinvasive and invasive lesions are more prevalent in chronically immunocompromised women, eg in HIV (Maimen et al-1998) and in renal transplants (Alhoub et al-1988 and Mayemon et al-1981)

8. Parity

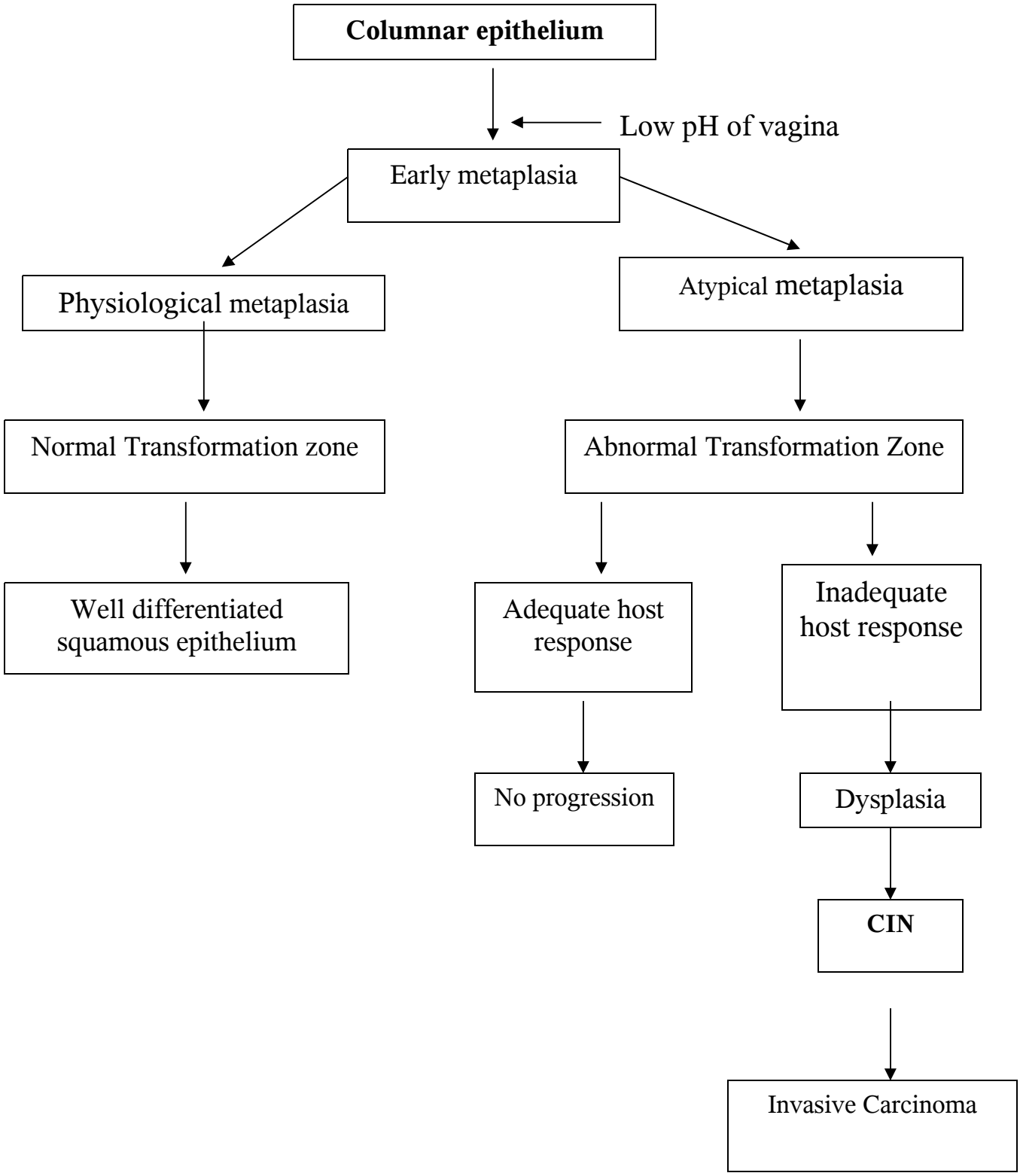
Although multiparity is associated with increased incidence of carcinoma cervix, there is no significant evidence to prove it. (Cramer et al, 1986)



The transformation to premalignant changes is a rapid process that lasts for days or weeks at the most, but promotion of these changes is a long term process. Some lesions can stay on the cervix indefinitely without change and some can progress quickly to CIN or invasive carcinoma depending on host response.

Scheme of pathogenesis of cervical neoplasia from STAFLA

MATTINGLY RF. AMJ. O.G. 1977



CIN II and CIN III require treatment. This is based on meta analysis showing that CIN II progresses to CIS in 20% of cases and to invasion in 5%

CIN	Regress	Persist	PROGRESS TO CIN III	Progress to Invasive CA
CIN I	57%	32%	11%	1%
CIN II	43%	35%	22%	5%
CIN III	32%	56%		> 12%

From Ostor AG-Inter.J.Gynaecol, Pathology, 1993; 12)

Precursors of invasive squamous cell carcinoma

The term dysplasia was first introduced by Reagon in 1953. Dysplasia represents a change causing an alteration and disorderly arrangement of the differentiated basal cells of stratified squamous epithelium.

The three degrees of dysplasia mild, moderate, severe according to Novak and Woodruff (1979) are characterized by,

I) Mild

The dysplastic cells extend from one quarter to one third of the way from the basal layer.

II) Moderate

Cellular aberrations extend through one half to two third of the thickness of epithelial layer.

III) Severe

The dysplasia cells penetrate through 75-90% of the epithelium.

Histopathology appearance of severe dysplasia resembles carcinoma in situ with the exception that a few cell layers near the surface are still capable of maturation.

Carcinoma in situ is characterized by malignant changes involving the whole thickness of squamous epithelium from the basement membrane to the surface, disclosing an immature disorganized pattern with complete loss of parity.

Richart (1966) first introduced the term cervical intra epithelial neoplasia (CIN) to denote the ranging degrees of intra epithelial abnormalities. The varying degree of CIN ranging from mild dysplasia to carcinoma in situ represents a continuum in the neoplastic process.

Richart (1966) graded the various degrees of CIN into

CIN I-Mild dysplasia

CIN II-Moderate dysplasia

CIN III-Severe dysplasia and carcinoma in situ

Carcinoma in situ has been grouped under CIN, although histologically there is a slight difference between severe dysplasia and carcinoma in situ.

Therapeutically and prognostically there is hardly any difference between the two.

Feature variations with increasing severity of dysplasia

Decrease	Increase	Varies
Cellular cohesion	Mitosis	Nuclear hypertrophy
Amount of cytoplasm	Nuclear cytoplasmic ratio	Anisokaryosis
Multinucleation	Annisochromatism	Hyperchromatism
Degree of maturation	Nuclear membrane irregularities	Nucleoli
Normal flora		

Screening procedures for CIN

1. Pap smear
2. Colposcopy
3. Cervicography
4. Speculoscopy (VIA, VILI)
5. HPV DNA detection and typing
6. Polarprobe (Truscan)
7. Spectroscopy

Pap smear

The Pap smear initially described by Papaniculaou and Traut is used to detect exfoliative cells from the cervix that may be precancerous or cancerous

Conventional Pap smear

According to American cancer society, ideal time to take Pap smear is 5 days after menstruation. The patient should be instructed not to use vaginal douche or any type of lubricant or spermicide for 24 hours prior to having a cytologic specimen obtained. The ectocervix and the area of vagina adjacent to the cervix must be fully visible when the smear is obtained. The patient should not bleed and should not have marked vaginal infection. The infection must be treated accordingly and patient must be rescheduled for Pap smear during next cycle.

The smear is taken with Ayer's spatula or with moistened brush around the external os by making 360º rotation with minimal pressure and smeared and fixed in a glass slide with 95% alcohol. Staining done with papanicolaou staining procedure which employ,

- Hemotoxylin as a nuclear stain and
- Orange G-6 Eosin alcohol (Eo-36) as cytoplasmic counterstains.the slides are finally mounted in Canada balsam and examined and interpreted.

Various forms of interpretations

Papanicolaou	Richart	WHO(1975)	BETHESDA (1998)
Gr I-normal cells	normal	Negative for cancer	normal
Gr-II-slightly abnormal, suggestive of inflammatory changes. repeat smear after treating infection	negative	Atypical squamous cells	Inflammatory HPV ASCUS
Gr-III-More,serious type usually indicative of biopsy	CIN I	Mild dysplasia	Low SIL
Gr-IV-Distinctly abnormal and definitely require biopsy	CIN II CIN III	Moderate and severs dysplasia	High SIL
Gr V- SCC	SCC	SCC	SCC

Cervical biopsy

Gold standard confirmatory test. Different types of biopsy methods are done which include punch biopsy, loop excision biopsy and conisation biopsy. Punch biopsy is the most commonly practiced method but it tends to crush and may not include stroma.A low voltage diathermy loop biopsy requires more sophisticated equipment but can control hemorrhage and produce samples of greater size. Prendiville et al, have shown that the artefactual damage is minimal and a larger biopsy can be taken for the diagnosis of micro invasion or invasion.

Screening intervals

ACS-American cancer society 2002 guidelines

1. Age to initiate screening: three years after the onset of sexual activity, not later than the age 21.

2. Screening frequency: annually with conventional cytology or every 2 years with liquid based cytology. After the age of 30, women with 3 consecutive normal tests may be screened every 2-3 years

3. Screening after hysterectomy: no cytologic testing after total hysterectomy for benign conditions

4. Discontinuation: After the age 70

5. Routine screening for HPV infection: not yet FDA approved. It approved, conventional or liquid based cytology combined with test for DNA from high risk HPV types should be performed not more than every 3 years.

Indication for colposcopy :

- Suspicious looking cervix
- Invasive carcinoma, CIN 2, CIN 3 on cytology
- Persisting CIN 1 abnormalities on cytology
- Infection with HPV
- VIA / VILI positive patients.

REID'S COLPOSCOPIC INDEX

Feature	0 points	1 point	2 points
Colour of acetowhite (AW) area	Low-intensity aceto whitening snow-white, shiny AW ; indistinct AW ; transparent AW ; AW beyond the transformation zone	Grey white AV with shiny surface	Dull, oyster-white ; Grey
AW lesion margin and surface configure tion	Feathered margins ; angular, jagged lesions ; flat lesion with indistinct margins ; microcondylomatous or micropapillary surface	Regular lesions with smooth, straight outlines	Rolled, peeling edges ; internal demarcations (a central area of high grade change and peripheral area of low grade change)
Vessels	Fine / uniform vessels ; poorly performed patters of fine punctuations and / or fine mosaic ; vessels beyond the margin of transformation zone; fine vessels within microcondylomatous or micropapillary lesions	Absent vessels	Well defined coarse punctuation or coarse mosaic
Iodine staining	Positive iodine uptake giving mahogany brown colour ; negative update of lesions scoring 3 points or less on above three categories	Partial iodine uptake by a lesion scoring 4 or more points on above three categories variegated, speckled appearance	Negative iodine uptake by a lesion scoring 4 or more points on the above three criteria
Scoring : A score of 0 to 2 points – Likely to be CIN 1 ; 3-4 points – overlapping lesion : likely to be CIN 1 -2 ; 5 to 8 points – likely to be CIN 2-3 lesions.			

Pathological basis of VIA

Application of 5% acetic acid dissolves mucus, Induces intra cellular dehydration, cause a reversible coagulation (or) precipitation of cellular protein. Thus the effect of acetic acid depends on the amount of cellular protein present in the epithelium.

Areas of increased nuclear activity and DNA content exhibit the most dramatic white color change.

When acetic acid is applied to normal squamous epithelium, little coagulation occurs in the superficial cell layer, as this is sparsely nucleated.

Although the deeper layer contains more nuclear protein, the acetic acid may not penetrate sufficiently and hence the resulting precipitation is not sufficient to obliterate color of underlying stroma. Areas of the CIN and invasive cancer undergo maximal coagulation due to their higher content of nuclear protein.

The acetowhite appearance is not unique to CIN and early cancer. It is also seen in other conditions like immature squamous metaplasia, in healing & regenerating epithelium, Leukoplakia, Condylomata when increased nuclear proteins are present.

BASIS OF VILI

1) Squamocolumnar epithelium contains glycogen whereas precancerous lesions & invasive cancer contain little or no glycogen

2) Iodine is glycophilic and is taken up by the squamous epithelium, staining it mahogany brown or black.

3) Columnar epithelium does not change color as it has no glycogen

4) Immature metaplasia, a red inflammatory lesion are at most only partially glycogenated &upon stained appear as scattered, ill defined uptake areas.

IARC criteria for interpretation of VIA and VILI

VIA Positive:

- Well defined**,** sharp, distinct, dense acetowhite area with or without raised margins, abutting the squamocolumnar junction in the transformation zone.
- Strikingly dense acetowhite area in the columnar epithelium
- Condyloma & leukoplakia occurring closer to the squamo columnar junction turning white after application of acetic acid.

VIA Negative:

- No acetowhite lesion on the cervix
- Polyps protruding from the cervix with blush-white acetowhite areas.
- Nabothian cyst appearing as button-like areas, whitish areas or pimples.
- Faint line like or ill-defined acetowhitening at squmocolumnar junction
- Shiny, digitative, inkish-white, cloudy white, bluish white, faint patchy, or doubtful lesion with ill defined indefinite margin blending with the rest of cervix
- Angular, irregular, digitations, acetowhite lesion resembling geographical region far away from the transformation zone.
- Ill defined, patchy, pale acetowhite areas in the inflamed unhealthy, ulcerated cervix with bleeding& mucopurulent discharge.
- Streak like acetowhitening in the columnar epithelium.
- Dot like areas in the endocervix, which are due to grape like columnar epithelium staining with acetic acid.

VILI POSITIVE:

Dense, thick, bright mustard yellow or saffron yellow iodine non-uptake areas abutting the squamocolumnar junction in the transformation zone. Precancerous lesions & invasive cancer do not take up iodine, as they lack glycogen and appear as well defined thick, mustard or saffron yellow areas.

VILI NEGATIVE:

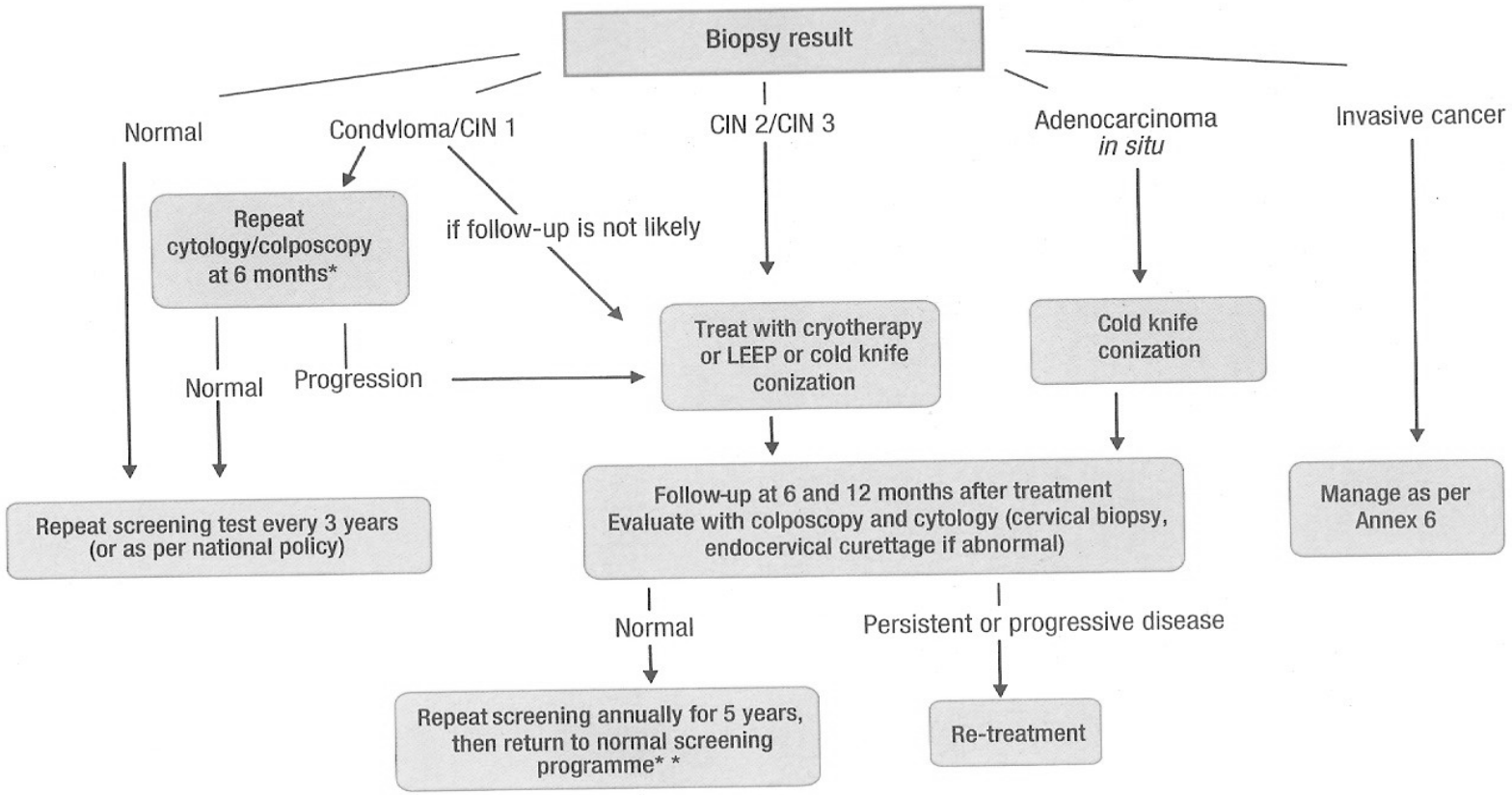
- Normal cervix where squamocolumnar epithelium turns mahogany brown or black and the columnar epithelium does not change color. no yellow areas seen.
- In ectropion, where an extensive areas of columnar epithelium with regular margin on the ectocervix remains without color change.
- Patchy, indistinct, ill defined, colorless or partially brown areas are seen in the cervix.
- None or partial Iodine uptake, pale areas corresponding nabothian follicles and (or) polyps are seen.
- Stippling (or) leopard skin appearance associated with T.VAGINALIS infection.

Pepper like non-iodine uptake areas seen in the squamous epithelium far away from squamocolumnar junction.

When satellite, thin yellow, non-iodineuptake areas with angular (or) digitating margin resembling geographical areas are seen far away from squamocolumnar junction.

Test	Strengths	Limitations	status
Conventional cytology	• History of long use • Widely accepted • Permanent record of test • Training and mechanism for quality control established • Modest investments in existing programmes can improve services • High specificity	• Results not immediately available • Systems needed to ensure timely communication of test results and follow up of women • Transport required for specimen to laboratory and for results to clinic • Requires laboratory quality assurance • Moderate sensitivity	• Available in many countries since the 1950s • Cytology based programmes have reduced cancer mortality in developed countries
Visual methods (VIA and VILI)	• Relatively simple and inexpensive • Results available immediately • Can be performed by wide range of personnel after short training • Low level of infra structure required • Can be combined with offer of immediate treatment in single visit approach	• High provider variability • Lower specificity resulting in high referral rate and over treatment • No permanent record of test • Not appropriate for post menopausal women • Lack of standardization • Frequent retraining needed	• Limited evidence available • Recommended for screening in low resource settings • Large randomized controlled trails under way to determine effect on cancer incidence and mortality

STANDARD MANAGEMENT OF CERVICAL PRECANCER



* If the lesion persists, the colposcopy should be repeated every 6 months until regression or progression occurs

** In case of CIN1 or CIN2, return to normal screening programme after 1 year

MATERIALS & METHODS

This cross sectional study was carried out in the gynecology OPD from September 2007 to October 2008.

A total of 250 women without history of active STD, Cervical neoplasia in the age group of 20-45 years were examined.

Women were considered eligible if they met all of the following requirement

1. Were aged between 20-45 years
2. No previous surgical procedure on the cervix or corpus
3. Had no history of abnormal Pap test in the past year.
4. Did not have any confirmed (or) clinically suspected immunosuppression

Cases referred from PHCs as a part of screening programme were also eligible.

Exclusion criteria:

- Were aged < 20 yrs
- Unmarried
- H/O Hysterectomy
- Previous surgical procedures on cervix
- Gross tumor on cervix
- Pregnancy

An oral questionnaire was administered to all women pertaining to age, parity, education, socioeconomic status, contraception history, chief gynaecologic complaints.

They were examined using an unlubricated–bivalve cusco speculam.

The cervix was exposed properly and excessive discharge when present was gently wiped away using a saline soaked cotton swab.

Cervix was closely inspected for macroscopic abnormalities such as erosion, polyp, congestion, nabothian cyst, ulceration, growth.

First Pap smear was taken using Ayer's spatula.

Then visual inspection with 5% acetic acid was done, 1 minute after application. Categorization was made as VIA Positive/Negative based on the criteria laid down by IARC.

After VIA, Lugol's Iodine was applied to the cervix with help of cotton swab suck and naked eye examination was done. Categorization was done as Positive or Negative based on IARC Criteria (Positive Pap Smear)

VIA&VILI Positive women were subjected to colposcopically directed cervical biopsy in the same sitting. But for Pap smear

positive cases. Colposcopically directed biopsy was done in the next visit after seeing the results.

Careful examination of cervix and the transformation zone was carried out approximately 1 minute after applying 5% acetic acid in the entire cervix.

Acetowhite epithelium, Punctuation, Mosaic pattern, Iodine negativity & atypical vessels prompted colposcopicaly targeted punch biopsies.

Cervical biopsies were fixed in formalin. Embedded in paraffin and processed into 5µm-thick haemotoxylin/eosin-stained section for light microscopy-following the routine diagnosis.

Thus, positive pap smear VIA&VILI patients were subjected to Colposcopically directed Biopsy & forwarded for Histopathology which was reviewed by Pathologists from Department of Pathology, Madurai Medical College as was the Pap smear for cytology. The reports were available within two weeks of the Pap smear or biopsy being taken.

Statistical analysis:

- Cervical biopsy report was considered as the gold stanadard
- Mild dysplasia was considered as positive-
- Sensitivity, specificity, positive and negative predictive values-were calculated for all screening tests-by using the cervical biopsy reports as the gold standard.

RESULTS

Table – 1 - AGE DISTRIBUTION :

Age in years	Screened		Positive	
	Frequency (n=250)	Percentage	Positive	Percentage
20 – 24	36	14.4	-	-
25 – 29	37	14.8	1	14.4
30 – 34	77	30.8	-	-
35 – 39	64	25.6	3	42.8
40 – 45	36	14.4	3	42.8
Total	250	100%	7	100 %
	Mean - 32.44 yrs		Mean - 38.21 yrs	

Among the 250 women screened

14.4% were between 20 – 24 yrs

14.8 % were between 25 – 29 yrs

30.8 % were between 30 – 34 yrs

14.4% were between 40 – 45 yrs

25.6% were between 35 – 39 yrs

Among those screened positive (out of 7 cases) 6 were in the age group of 35-45 yrs. Mean age of the women screened was 32.44 yrs and Mean age of those screened positive was 38.21 yrs.

Table – 2 - EDUCATIONAL STATUS :

Education	No.of cases (n=250)	Percentage
Illiterate	103	41.2%
Upto 10^{th} standard	123	49.2%
+1/+2/Degree	24	9.6%

Among the 250 women studied, 46.2% (103/250) were illiterate, 49.2% (123/250) had high school education and 9.6% (24/250) had higher education.

Table – 3 - SOCIO-ECONOMIC STATUS :

Income per month	No.of cases (n=250)	Percentage
I < 1000	142	56.8 %
II 1000 – 1500	46	18.4 %
III 1500 – 2000	52	20.8 %
IV > 2000	10	4 %

Majority, 56.8% of women belonged to low income group (< Rs. 1000 per month)

TABLE – 4 : AGE AT MARRIAGE

Age at marriage	No.of cases (n=250)	Percentage	Dysplasia (n=7)	%

< 15 yrs	33	13.2	-	-
15-20 yrs	121	48.4	5	71.43%
20-25 yrs	73	29.2	2	28.57%
25-30 yrs	13	5.2	-	-
> 30 yrs	10	4.0	-	-
Total	250	100%	7	100%

Mean age at marriage - 19.42 years

48.4% (121/250) of those screened were married between the age of 15 – 20 years. The mean age at first intercourse was 19.42 years.

71.43% (5/7) of those diagnosed with CIN were married between the age of 15-20 yrs.

28.57% (2/7) of those diagnosed with CIN were married between the age of 20-25 yrs.

Table - 5 : Duration of Marital life :

Duration years	Cases		Dysplasia	
	Frequency (n=250)	Percentage		Percentage
< 5 yrs	45	18	-	-
5 – 10 yrs	73	29.2	1	14.28
11 – 20 yrs	83	33.20	4	57.14
> 20 yrs	49	19.6	2	28.56

In the study group, 18% had < 5 yrs of marital life.

29.2% had 5-10 yrs of marital life

33.2 % had 11-20 yrs of marital life and

19.6% had marital life of > 20 yrs.

Table – 6 : Multiple Sex Partner

> 1 partner	No.of cases (n=250)	Percentage
Yes	4	1.6 %
No	246	98.4 %

In the age group - 1.6% had contact with more than 1 partner, Most of the patients gave no history of treatment for STDs.

None of the screen positive patients gave h/o of contact with more than one partner

Table - 7 : PARITY

Parity	Cases		CIN	
	Frequency (n=250)	Percentage	Frequency	Percentage
1	29	11.6	-	-
2	106	42.4	2	28.57
3	97	38.8	4	57.14
4	18	7.2	1	14.28
Total	250	100	7	100 %

Among the 250 women studied

11.6% were primipara, 42% were para2, 38.8% were para 3 and 7.2% were para 4 and above.

In the screen positive group, majority (5/7), 71% of them were para 3 and above.

Table - 8 : CONTRACEPTION

Contraception	Cases		Dysplasia	
	Frequency (n=250)	Percentage	Frequency (n=7)	Percentage
Barrier	21	8.4	-	-
OCP	3	1.2	-	-
IUCD	18	7.2	1	14.28
Permanent sterlization	160	64.2	4	57.14
No contraceptive	48	19.2	2	28.57

Among those 250 women studied, 8.4% practiced barrier method, 1.2% were taking oral contraceptive pills, 7.2% had IUCD inserted, 64% of them were permanently sterilized, 19.2% did not adopt any form of contraception. 1 case (1/7) of mild dysplasia had Cu-T inserted 4 ½ years back. 4 of the positive cases (4/7) were permanently sterilized. 2 of the positive cases (2/7) did not practice any form of contraception.

Table – 9 : Complaints

Complaints	No.of cases (n=250)	Percentage	Dysplasia (n=7)	%
White discharge	174	69.6	4	57.14
Lower abdominal pain	32	5.6	-	-
Low back ache	18	4.8	1	14.28
Post coital bleeding	14	12.8	2	28.56
Intermenstrual bleeding	12	7.2	-	-

In the study group, predominant or chief complaints were white discharge and lower abdominal pain. 69.6% of the cases had white discharge. 20% had abdominal pain and low back ache. Only 5.6% of the patients had post coital bleeding. Intermenstural bleeding was seen in 7.2% of the cases.

Of those diagnosed with dysplasia, white discharge was the predominant complaint. 57.14% (4/7) of those diagnosed with dysplasia gave history of white discharge. 28.56% (2/7) had post coital bleeding as the predominant complaint. 14.28% (1/7) gave history of low backache.

Table – 10 : Clinical appearance of Cervix :

Appearance of Cervix	No.of cases (n=250)	Percentage	Dysplasia (n=7)	%
Normal	54	21.6	-	-
Ectopy cervix	132	52.8	5	71.43
Hypertrophy and congestion	17	6.8	2	28.57
Nabothiancyst	43	18.0	-	-
Cervical polyp	2	0.8	-	-

When the cervix was visualized with cuscos speculum, cervix was healthy in 21.6 % of the patients and unhealthy in rest of the patients.

Ectopy cervix was seen in 52.8% of cases 17.0% of the patients had nabothian cyst.

Hypertrophy with congestion was present in 6.8% of cases. Cervical polyp was seen in 0.8% of the study subjects.

In 2 cases with severe dysplasia, cervix was hypertrophied, congested and badly eroded.

Table – 11 : Papsmear Results :

Appearance of Cervix	No.of cases (n=250)	Percentage
Normal	64	25.6
Inflammatory	178	71.2
Mild dysplasia	5	2.1
Moderate dysplasia	1	0.4
Severe dysplasia	2	0.8
Total	250	100

25.6% (64/250) of the patient had normal papsmear. 71.2% (178/250) of the study group had inflammatory smear.

2.1% (5/250) of the patients had mild dysplasia.

0.4% (1/250) of the patients had moderate dysplasia.

0.8% (2/250) of the patients had severe dysplasia

Table – 12 : Result of VIA:

VIA	No.of cases (n=250)	Percentage
VIA positive	75	30%
VIA Negative	175	70%

VIA was positive in about 30% of the study group and it was negative in 70% of the study subjects.

Table – 13 : Result of VILI :

VILI	No.of cases (n=250)	Percentage
Positive	63	25.2%
Negative	187	74.8%

VILI was positive in 25.2% of the patients and it was negative in about 74.8% of the patients.

Table – 14 : Biopsy Report

Biopsy Report	No.of cases	Percentage
Chronic cervicitis	65	76.4 %
Reactive squamous metaplasia	9	10.5 %
Hyperplasia of squamous epithelium	4	4.7%
Mild dysplasia	3	3.6%
Moderate dysplasia	1	1.2%
Severe dysplasia	3	3.6%
	85	100 %

Of the study population, 76.4% were diagnosed to have chronic cervicitis. Reactive squamous metaplasia was present in 10.5% of the patients. Hyperplasia of squamous epithelium was present in 4.7% of the patients. 3.6% of the patients had mild

dysplasia. Moderate and severe dysplasia was present in about 1.2% and 3.6% of the patients respectively.

Table – 15 : Correlation of Papsmear with biopsy cervix

Papsmear	Chronic cervitis	Meta plasia	Hyper plasia	Mild dysplasia	Moderate dysplasia	Severe dysplasia
Normal	10	3	2			
Inflammatory	52	6	2	2		
Mild	3			1		1
Moderate					1	
Severe						2
	65	9	4	3	1	3

2 cases reported as inflammatory smear by cytology had mild dysplasia in cervix biopsy

3 cases reported as mild dysplasia had chronic cervicitis

1 patient reported as mild dysplasia had severe dysplasia

1 patient reported as moderate dysplasia had same findings in biopsy cervix

2 patients reported as severe dysplasia were also found to have severe dysplasia in HPE report.

Correlation between papsmear and biopsy report for lesions diagnosed as moderate and severe dysplasia was good.

Table : 16 Result of Screening Test compared with Final Disease status established by reference standard

	Normal	Mild dysplasia	Moderate dysplasia	Severe dysplasia	Total
VIA					
Positive	69	2	1	3	75
Negative	174	1	0	0	175
VILI					
Positive	56	3	1	3	63
Negative	187	0	0	0	187
Cytology					
Negative	240	2	0	0	242
Positive	3	1	1	3	8

Table – 17 Calculation of Sensitivity, Specificity, PPV, NPV

	VIA	VILI	Papsmear
True Positive	6	7	5
False Positive	69	56	3
True Negative	174	187	240
False negative	1	-	2
Sensitivity - $\frac{TP \times 100}{TP\& FN}$	85.7 %	100%	71.4%
Specificity - $\frac{TN \times 100}{TN\& FP}$	71.6%	76.9 %	98.7 %
PPV – $\frac{TP \times 100}{}$	8.2%	11.11%	62.5%

TP & FP			
NPV $\frac{\text{TN x 100}}{\text{TN + FN}}$	99.37%	100%	99.1%

The sensitivity, specificity, PPV, NPV of VIA are 85.6%, 71.6%, 8%, 99.37% respectively.

The sensitivity, specificity, PPV, NPV of VILI are 100%, 76.9%, 11.11%, 100% respectively.

The sensitivity, specificity, PPV & NPV of papsmear are 71.4%, 98.7%, 62.5%, 99.1% respectively.

Correlation between Papsmear and Visual Findings

Table - 18

Papsmear	VIA Positive	VIA Negative
Normal (242)	68	174
Abnormal (8)	7	1

Papsmear	VILI Positive	VILI Negative
Normal (242)	55	187
Abnormal (8)	8	-

Of the 250 cases screened, papsmear is abnormal in 8 patients.

Correlation between abnormal papsmear and visual findings was studied.

VIA was positive in 7 of the 8 abnormal papsmear cases

1 patient with papsmear abnormality was negative with VIA.

VILI was positive in all the 8 abnormal papsmear cases.

DISCUSSION

Cervical Cancer is the second most frequent cancer world wide in women after breast cancer. However, invasive cancer of cervix is considered to be preventable condition as it is associated with a preventive stage amenable to screening and treatment.

Mean age of the women screened was 32.44 years and mean age of those screened positive was 38.21 years. Dysplasia was found to be more common between 35-45 yrs. 6 of the 7 patients with dysplasia were in the age group of 35-45 years. Kustagi and Fernandes in their study showed the prevalence of dysplasia was higher in women over 30 years. Vaidya showed in his study that CIN was more prevalent in the age group > 35 years.

Regarding educational status, abnormal cervical lesions like chronic cervicitis, dysplasia and malignancies were common among illiterates. The same was confirmed in our study.

Socioeconomic status had always been playing an epidemiological role in genesis of dysplasia. In our study, chronic cervicitis and dysplasia were common in low income group. Poor personal hygiene, poor living conditions and early age at first

intercourse are factors associated with both low socio economic status and cervical cancer.

Regarding parity, our study showed increased incidence of dysplasia among multiparous women. 71% of those screened positive were para 3 and above. The other two cases screened positive were para 2 one had mild dysplasia and the other subject had severe dysplasia. Kusthagi and Fernandez showed the prevalence of CIN was significantly higher in parity of > 2.

This might be attributed to hormonal and nutritional charges that occur in pregnancy, immunosuppression during pregnancy and cervical trauma during vaginal delivery. (Becker et al)

Incidence of dysplasia (85.6%) was found to be common in those married for more than 10 year (about 85.67%). Kushtagi et al, had demonstrated the severity of underlying CIN increased with increase in the duration of marital life.

1 patient with Cu-T had mild dysplastic changes in cytology and was proved with biopsy cervix. 57.14% of the cases screened positive for dysplasia were permanently sterilized.

Among the complaints, white discharge was present in majority of women with chronic cervicitis, dysplasia and metaplasia.

Excessive vaginal discharge playing a role in contributing to the development of CIN was also proved to be a risk factor in the study conducted by Vaidya et al. In their study, 24% had vaginal discharge.

On examination, majority of the patients had ectopy cervix (52.8%). In all patients with severe dysplasia cervix was badly eroded and was bleeding on touch.

Analysis of results :

The screen positivity rate for cytology was 3.3% and for VIA, VILI, it was 30%, 25.4% respectively.

High screen positivity rate for visual lesions could be attributed to large number of referral cases from other primary health centres as a part of screening programme. (Varumun kappom)

The sensitive of VIA, VILI and papsmear were 85.6 %, 100%, 71.4% respectively.

Thus VILI was found to have the highest sensitivity and detected all the true positive lesions.

The specificities of VIA, VILI and papsmear were 71.6%, 76.9% and 98.7%.

Thus papsmear was the most specific test. However, lower specificity of the visual test, means that larger number of women will need investigations and treatment.

The positive predictive value of VIA, VILI and papsmear were 8%, 11.11% and 62.5% respectively.

Papsmear was found to have the highest positive predictive value among the tests, meaning that papsmear correctly identified abnormal lesions.

The negative predictive value of all the tests exceeded 99%.

64 (25.6%) patients had normal papsmear. 178 patients (71.2%) of the study group had inflammatory smear. 2.1% of the patients had mild dysplasia, 1.2% of the patients had moderate and severe dysplasia.

Those with inflammatory smear were treated with antibiotics and subsequently followed up with repeat papsmear at a interval of 6 months.

2 patients reported as inflammatory smear by cytology were positive with VIA and VILI and subsequently taken biopsy cervix showed mild dysplasia.

5 (2.1%) patients had mild dysplasia by cytology. Among the 5 patients, 3 patients were found to have chronic cervicitis by biopsy cervix. 1 patient was confirmed to have mild dysplasia, 1 patient was found to have severe dysplasia. Correlation between papsmear and biopsy report for lesions diagnosed as moderate and severe dysplasia was good.

The cytology findings in this study are similar to the finding reported by J.J. et al (1997). Normal or inflammatory in 97%, 1.6% showed LSIL, 1% showed HSIL.

It would be inappropriate to compare our small findings with the studies, involving large population in series of trials, still this has been necessary as part of study. The sensitivity and specificity for cytology in the present study was 71.4% and 98.3% as compared to Sankaranarayan et al, 1998 with reported sensitivity of 86.2% and specificity of 91.3%.

In the recent review of the accuracy of cervical cytology based on published studies from developed countries, the average sensitivity for cytology ranged from 47% to 62% and specificity ranged from 60% to 95%.

The sensitivity and specificity of cytology, 73.4%, 98.7% respectively in this study were comparable to that of

Singh et al 2001 - Sensitivity – 73.3%
- Specificity – 99.0 %

Parashri et al 2000 - Sensitivity - 78.9%
- Specificity - 99.0 %

Addition of visual test with papsmear in parallel was found to increase the sensitivity of papsmear with a compromise in specificity.

Among the single test in our study, cervical cytology demonstrated the best balance of sensitivity, specificity and predictive value.

Results of VIA / VILI

30% of those screened were positive for VIA. 25.2% were positive for VILI.

The sensitivity, specificity, PPV and NPV for VIA are 85.6%, 71.6%, 8 %, 99.3% respectively and for VILI 100%, 76.9%, 11.1% and 100% respectively.

The sensitivity and specificity are comparable to Cecinni et al, with a sensitivity and specificity of 88% and 75% respectively. Thus VIA & VILI were more sensitive than papsmear for detection of preinvasive and invasive stage of cervical carcinoma as in other study by Denny et al. However, study by Sankaranarayanan et al,

reported equally comparable specificities for both tests, 93.2% for VIA and 92.7% for cytology which is not similar to our study. High number of false positive and consequently low specificity of VIA, could be due to large number of inflammatory lesion. Also majority of the cases were referred as VIA positive from other primary health centres.

The high NPV of both VIA and VILI warrants particular mention. The use of VIA / VILI as a primary screening test means that women assessed as test negative would be reassured most probably that they do not have high grade CIN or Cancer cervix. With regards to VIA, VILI and cytology, the results were consistent with recent study findings of India and Africa which have shown VIA / VILI are more sensitive than cytology. (Sankaranarayanan et al, 2004a).

Abnormal pattern in both VIA and VILI were common among those presented with abnormal cytology. One Papsmear positive case was missed by VIA. VILI diagnosed all abnormal papsmear case. The significant association of abnormal VIA and VILI with cytological abnormalities suggests that both tests have potential to detect abnormal cervical diseases.

SUMMARY

The present study was conducted in the Department of O&G, GRH, Madurai, from September 2007 to October 2008 involving 250 women. They were screened with papsmear, VIA & VILI and those positive with any of these tests were subjected to colposcopically directed biopsy cervix. The sensitivity, specificity, PPV and NPV were calculated using biopsy report as the reference standard.

- The mean age of the study subjects and those with screen positivity were 32.44 yrs and 38.21 yrs respectively
- Majority (85.6%) of the CIN patients were in the age group of 35-45 years
- The mean age at marriage was 19.42 years and majority of them screened positive were multi para.
- One patient with IUCD had mild dysplasia
- Majority (64.2%)of the patients studied were permanently sterilized

Among those attending the OPD, white discharge was the predominant complaint. Two patients with severe dysplasia gave H/o of post coital bleeding.

- Cervix was healthy in (21%) of the patients and unhealthy in rest of the patients.
- 2.1% of the patient had mild dysplasia, 1.2% of the patients had moderate and severe dysplasia. Screen positivity rate for papsmear was 3.2%.
- 30% of the patients had positive VIA.
- 25.2% of the patient were positive for VILI.
- Papsmear missed 2 cases of dysplasia and
- VIA missed 1 case of dysplasia

The sensitivity, specificity, PPV & NPV of VIA, VILI, Papsmear are as follows.

VIA – 85.6%, 71.6%, 8%, 99.37%

VILI - 100.6%, 76.9%, 11.11%, 100%

Papsmear - 71.4%, 98.7%, 62.5%, 99.9% respectively

Thus VIA, VILI were more sensitive than cytology.

However papsmear was more specific

As a single best, papsmear demonstrated the best balance of sensitivity and specificity and predictive value.

CONCLUSION

In conclusion , as a single test ,cytology was associated with the best balance of sensitivity, specificity and predictive values on the basis of our findings**.**

The significant association of abnormal VIA and VILI with cytological abnormalities suggests that both tests have potential to detect abnormal cervical diseases.

The result of current study indicate that VIA and VILI are simple objective test. The result of this procedures is available immediately, allowing an algorithm of further investigations to be carried out for the identification of cervical lesions.

VIA & VILI may find a place as an alternative low technology and low cost methods of screening and case finding. Further research is required to improve specificity without compromising sensitivity.

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PROFORMA

SCREENING WITH VIA , VILI, PAPSMEAR

1. Name : 2. OP / IP No. 3. Age

4. Address :

5. Education :

6. LMP : 7. Marital status

8. Age at marriage / first sexual intercourse

9. Total number of pregnancie / miscarriages

10. Do you suffer from the following ?

Excessive vaginal discharge — Itching in the external genitalia

Ulcer in the external genitalia — Lower abdominal pain

Pain during sexual intercourse — Bleeding during intercourse

Intermenstrual bleeding — Low backache

11. Visual examination findings :

- Cervical polyp
- Nabothian follicles
- Erosion cervix
- Leukoplakia
- Condyloma
- Growth

12. Papsmear

13. VIA findings

One minute after application of 5% acetic acid (VIA)

1. Positive 2. Negative

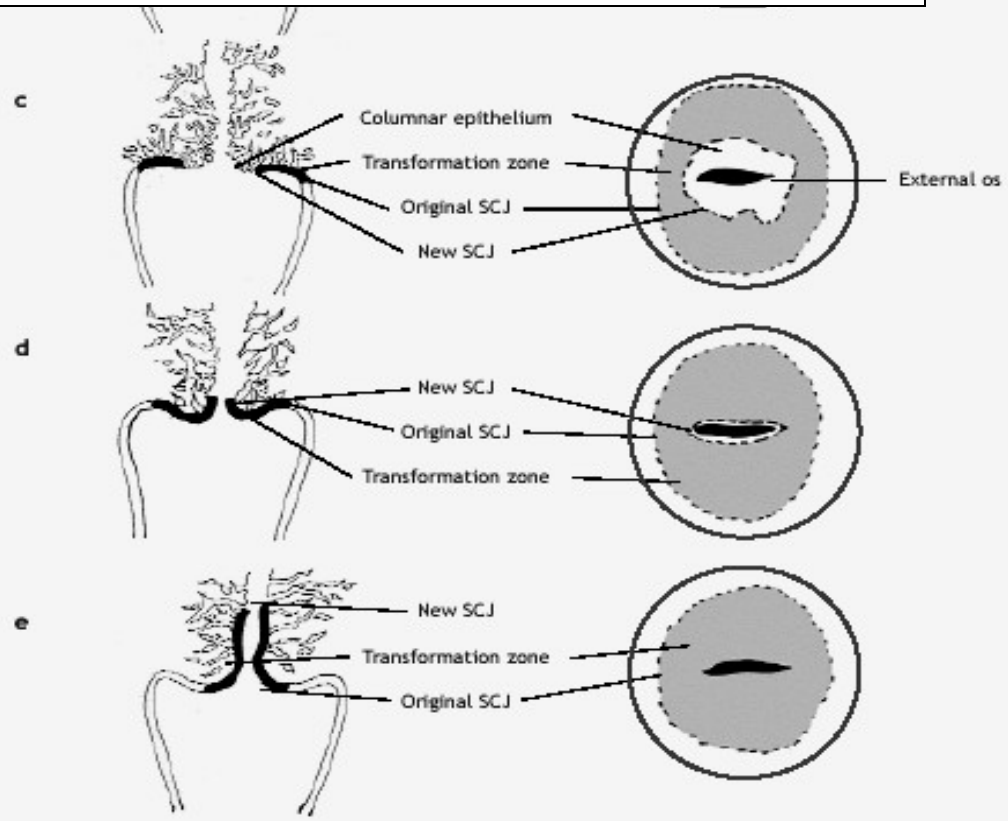
14. Findings after application of Lugol's Iodine (VILI)

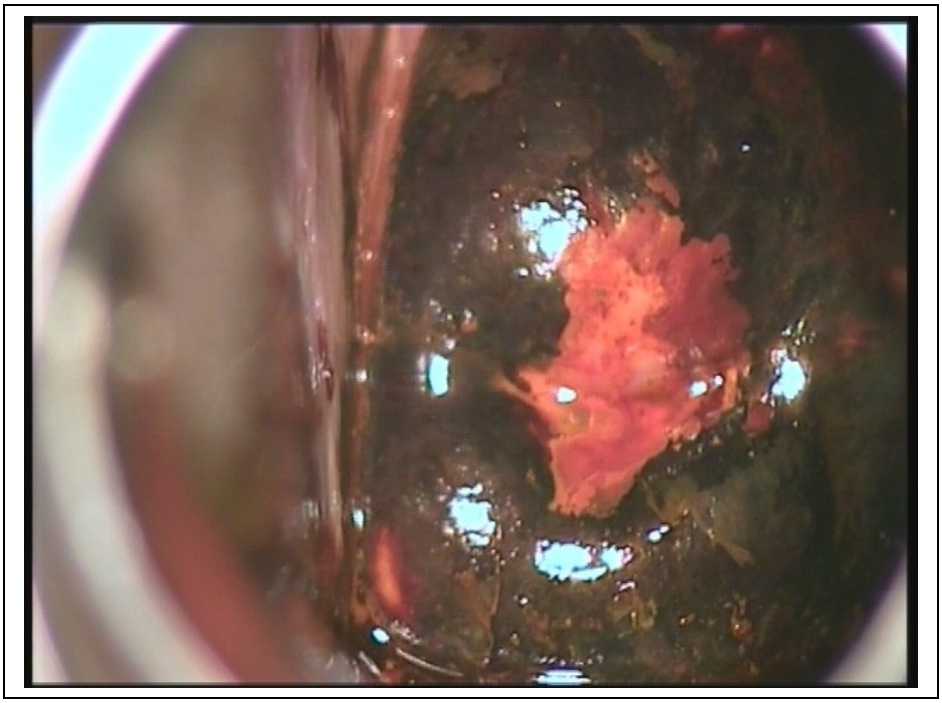
1. Positive 2. Negative

15. Biopsy cervix taken ? 1. Yes 2. No

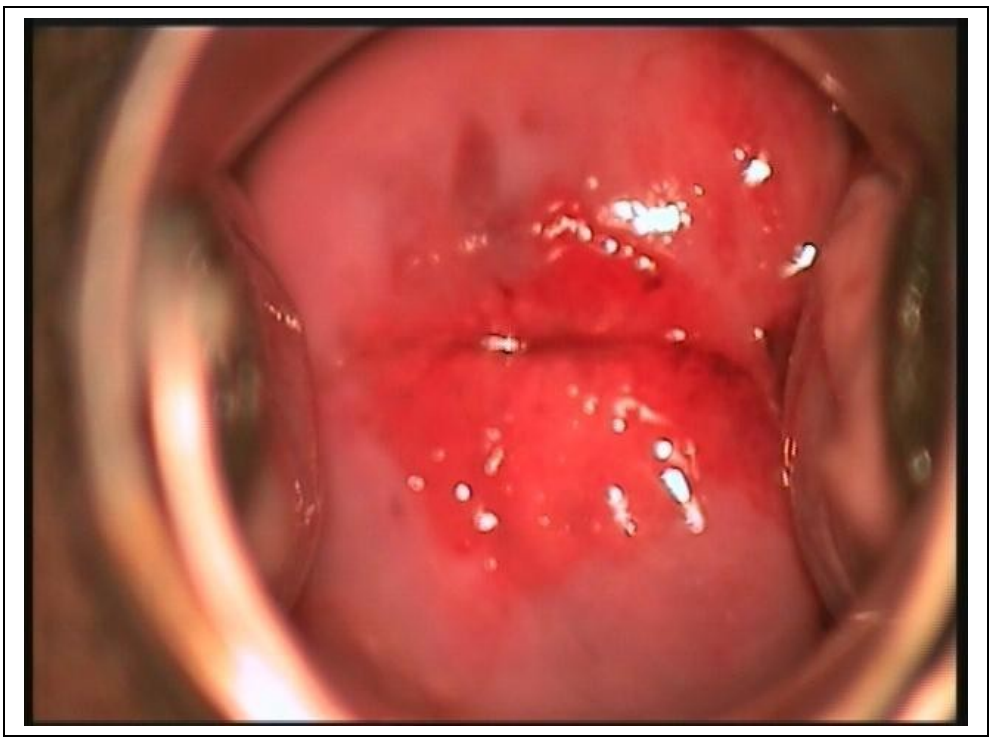
MASTER CHART ABBREVIATIONS

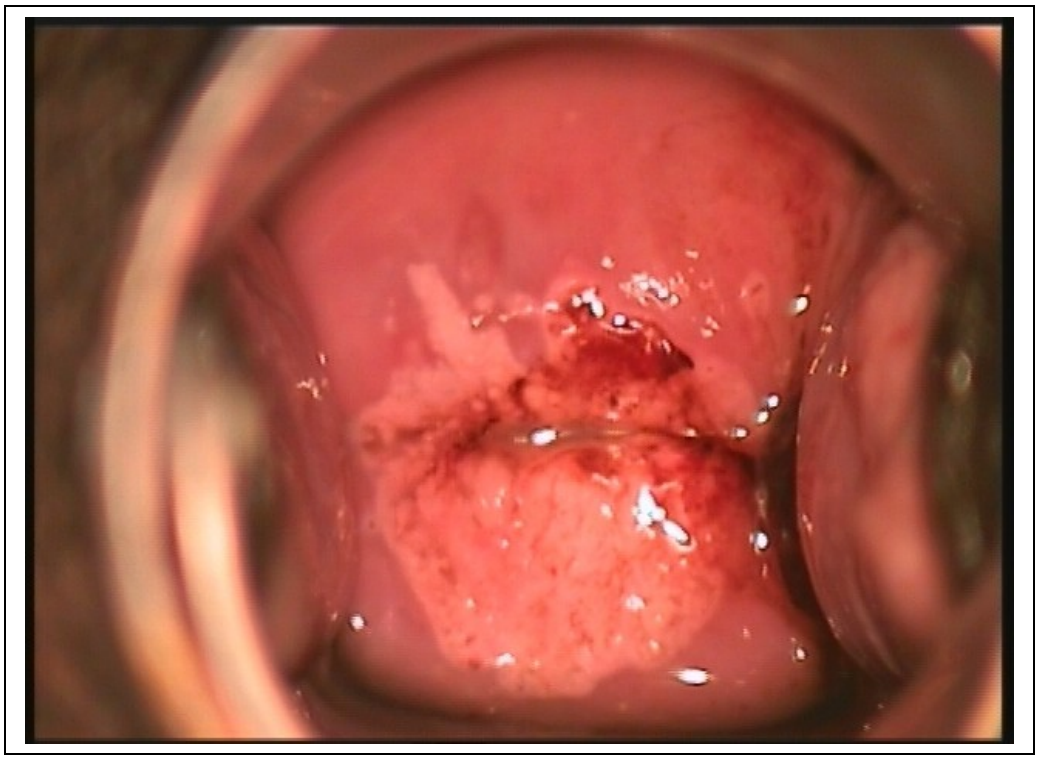
SE	Socio economic Status
PS	Permanent sterilization
Pain abd.	Pain Abdomen
White dis	White discharge
Post coital bld	Post coital bleeding
Hyper, con	Hypertrophied, congested cervix
Nab. Cyst	Nabothian cyst
Hyper, b/t	Hypertrophied, bleeds on touch


c
Columnar epithelium
Transformation zone
Original SCJ
New SCJ
External os
d
New SCJ
Original SCJ
Transformation zone
e
New SCJ
Transformation zone
Original SCJ

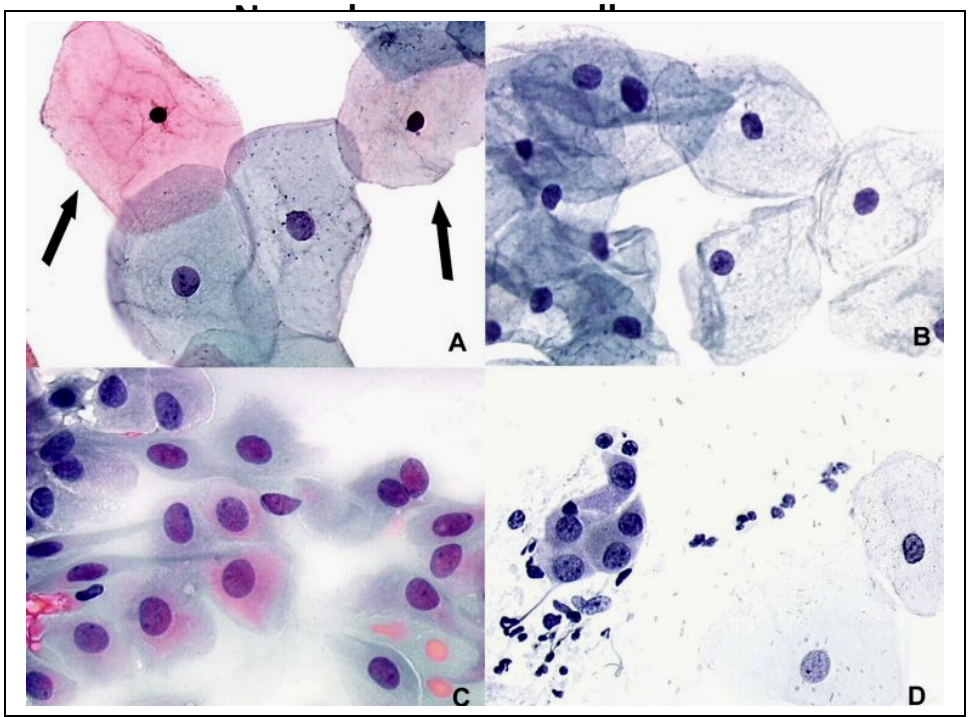


VILI NEGATIVE





VIA POSITIVE

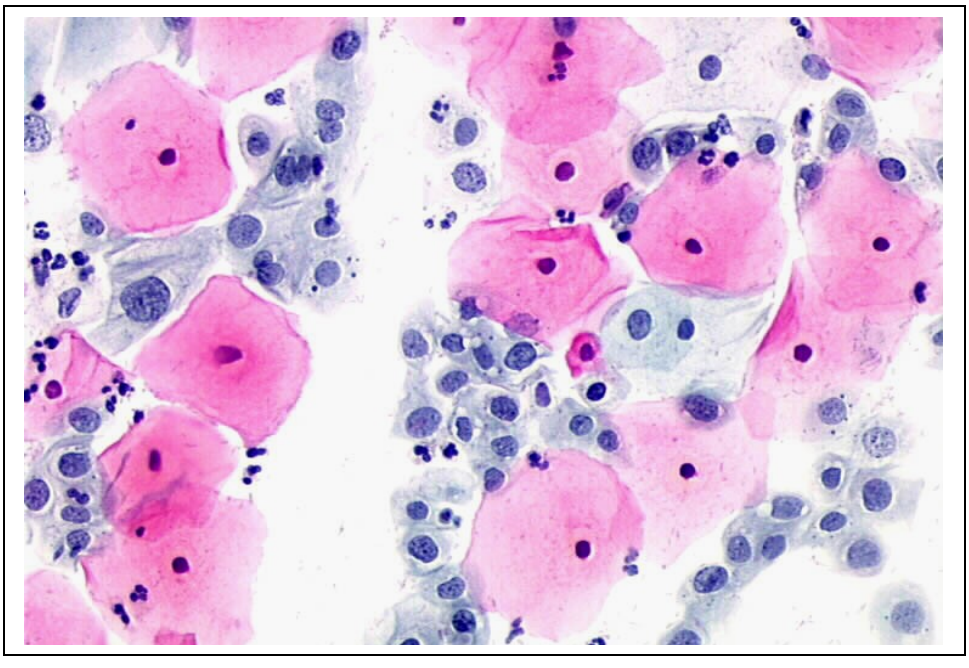


A: superficial cells (arrows); B: intermediate cells;
C: parabasal cells; D: metaplastic cells.

Low-grade Squamous Intraepithelial Lesion (LSIL)

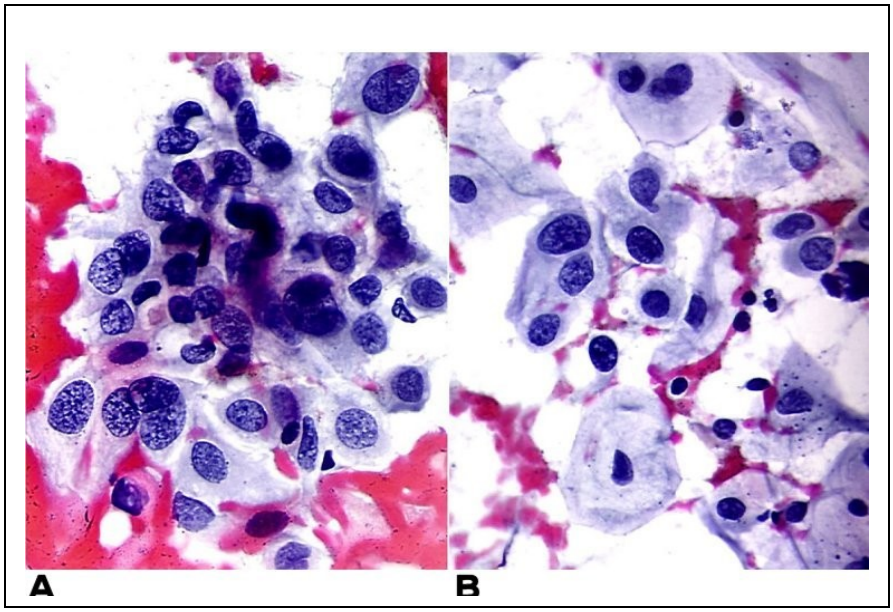
LSIL: eosinophilic squamous cells with a perinuclear empty cavity surrounded by cytoplasmic thickening and with moderate nuclear enlargement: typical koilocytes.

HSIL



Parabasal cells with nuclear enlargement, irregular nuclear outlines, with anisokaryosis and anisocytosis in a homogenous cell population.

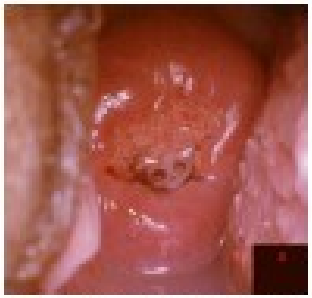
Squamous cell carcinoma



Group of moderately to poorly differentiated malignant cells, without keratinization

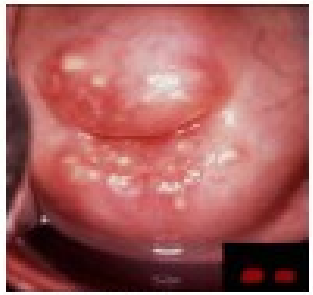
VIA NEGATIVE

No definite acetowhite area



Acetowhitening of the mucus on columnar epithelium

Mucus plug

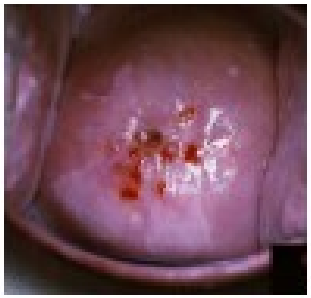


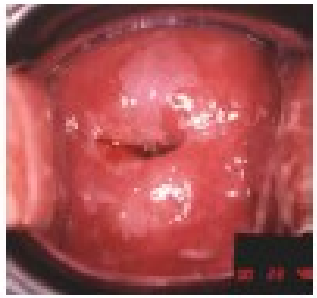
Nabothian cysts

Polyp

Acetowhite area far away from SCJ

VIA POSITIVE

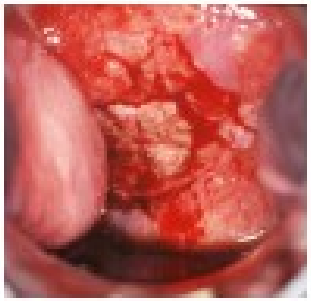




<----------- Well-defined, acetowhite lesions touching the SCJ or close to the os ----------->

Acetowhiteness on the entire cervix

CANCER



<---------- Acetowhitening of growth on the cervix ---------->

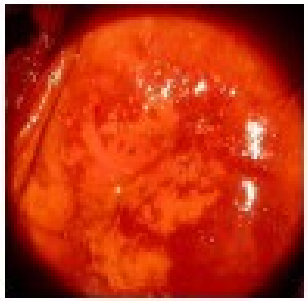
Acetowhitening of growth on the cervix; partly obliterated by bleeding

SCJ: Squamocolumnar junction

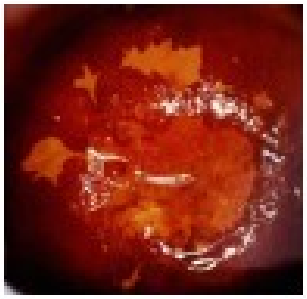
Source: R. Sankaranarayanan, Ramani S. Wesley. A practical manual on visual screening for cervical neoplasia (IARC technical publication No 41)
Available from: press@iarc.fr (IARC/Press)

VILI NEGATIVE

Black squamous epithelium. No colour change in columnar epithelium. No yellow areas

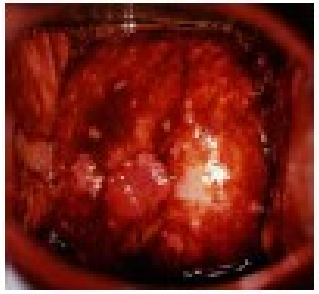


Patchy, cotton wool, scattered yellow areas indicating immature squamous metaplasia and inflammation



'Satellite' yellow areas away from SCJ

Pepper-like yellow areas due to inflammation away from SCJ

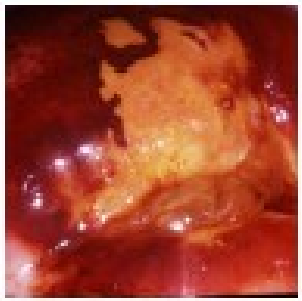


Pepper-like scattered yellow spots all over the Cx due to inflammation. No iodine uptake in the polyps

Leopard like appearance due to scattered yellow areas

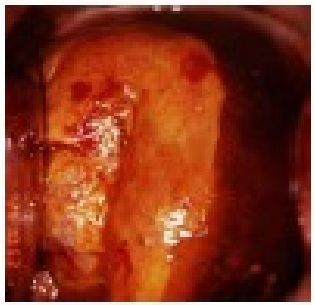
VILI POSITIVE

Well-defined yellow area touching the SCJ in the upper lip



Circum-orificial, large yellow areas extending into the canal

CANCER



Dense, thick, irregular yellow coloration of the growth on the Cx

SCJ: Squamocolumnar junction

Source: R. Sankaranarayanan, Ramani S. Wesley. A practical manual on visual screening for cervical neoplasia (IARC technical publication No 41)
Available from: press@iarc.fr (IARCPress)